FLUORINE CHEMICAL SHIFTS IN COMPLEXES OF SODIUM TRIFLUORALKYLSULFATES WITH REDUCED PROTEINS[1]

Michael L. Smith[2] and Norbert Muller[3]

Department of Chemistry, Purdue University
West Lafayette, Indiana 47907

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Summary: Equilibrium dialysis experiments were carried out for several proteins, reduced with dithioerythritol, in aqueous buffer and the anionic surfactants, sodium 12,12,12-trifluorododecylsulfate or sodium 13,13,13-trifluorotridecylsulfate, with surfactant concentrations above the critical micelle concentration. Fluorine chemical shifts were determined for each retentate and dialysate solution. The results show that most of the proteins bind 3.2 ± 0.4 millimoles of fluorinates surfactant per gram. In every case the chemical shift of the bound detergent ions is very near that found for micelles, suggesting that the bound ions form micelle-like aggregates.

Introduction. Previous work from this laboratory showed that although F_3SDS[4] is slightly less hydrophobic than ordinary SDS, the two detergents act rather similarly as regards micelle formation (1), binding with native bovine serum albumin (2), and complex formation with polyethylene glycol (3). The trifluoromethyl groups of F_3SDS produce a fluorine nuclear magnetic resonance signal with a chemical shift that is subject to large variations depending on whether their environment consists of water, micellar material, or organic solvent, thus providing information about the nature of the immediate surroundings of these groups. In view of current interest in complexes between SDS and reduced, denatured proteins (4-7) it seemed desirable to use magnetic resonance and equilibrium dialysis measurements to determine whether or not F_3SDS forms similar complexes and, if so, to extract as much structural information as possible from the bound-detergent chemical shifts.

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2. National Science Foundation predoctoral trainee.
3. To whom correspondence should be addressed.
4. Abbrevations: F_3SDS, sodium 12,12,12-trifluorododecylsulfate; SDS, sodium dodecylsulfate; F_3STS, sodium 13,13,13-trifluorotridecylsulfate, cmc, critical micelle concentration.

Materials and Methods. The preparation and properties of F_3SDS and F_3STS have been described elsewhere (1,2,8). 1,4-Dithioerythritol was purchased from Cyclo Chemical and used without further purification. β-Lactoglobulin A was a gift from Prof. J. F. Foster and was desalted on a G-25 sephadex column and lyophilized before use. Other proteins were commercial preparation from sources identified in Table I and were used as received. Solutions were prepared in sodium phosphate buffers with ionic strength 0.050 (F_3SDS experiments) or 0.10 (F_3STS solutions) at a pH of 6.8. Solutions of known protein concentration were prepared volumetrically from stock solutions which had been analyzed spectrophotometrically at appropriate wavelengths using published values of the required extinction coefficients (8). A five to tenfold excess of dithioerythritol, based on the number of disulfide bridges to be reduced, was added to each protein solution. Detergent was introduced as needed by taking a measured volume of a stock solution freshly prepared by weighing dry detergent into a volumetric flask and diluting with the buffer solution.

Dialysis experiments were conducted in plastic cells with 1 ml or 5 ml retentate and dialysate compartments separated by a cellulose membrane and rotated end-over-end at 20 r.p.m. at 35 to 38°. The retentate compartment initially contained protein and detergent, and the dialysate compartment contained detergent at a concentration somewhat below the anticipated equilibrium value. Preliminary experiments (8) with β-lactoglobulin A showed that under these conditions equilibrium was attained in 24 hours. In subsequent experiments with F_3SDS the cells were rotated for 48 to 62 hours, and it was assumed that this would suffice for equilibration. In the single experiment with F_3STS dialysis was continued for 120 hours.

Fluorine chemical shifts for the detergent in the retentate and dialysate solutions were measured at 35° (40° for the F_3STS solutions) with a Varian XL-100-15 nuclear magnetic resonance spectrometer operated at 94.077 MHz. Positive shifts are to high field from the external reference, 1,1,2-trichlorotrifluoro-1-propene. Chemical shifts were also measured for a series of solutions containing known amounts of F_3SDS or F_3STS in buffer with 0.4 g/l of dithioerythritol.

The specific optical rotation at 233 nm was determined at 35° for lysozyme at a concentration of 0.0139 g/dl in the presence of a tenfold excess of dithioerythritol and variable amounts of SDS or F_3SDS, using a Cary model 60 spectropolarimeter.

Results and Discussion. In each solution, rapid exchange of detergent anions among all sites leads to a single fluorine resonance signal with a time-averaged chemical shift, δ, which obeys

$$[D^-]_t\delta = [D^-]_f\delta_f + [D^-]_b\delta_b + [D^-]_m\delta_m. \qquad [1]$$

Here $[D^-]_f$, $[D^-]_b$, and $[D^-]_m$ stand for the concentrations of free, bound, and micellized anions, respectively, δ_f, δ_b, and δ_m are the corresponding chemical shifts, and $[D^-]_t = [D^-]_f + [D^-]_b + [D^-]_m$. When protein is absent and the total concentration exceeds the cmc by at least 10%, it is a good approximation to set $[D^-]_f$ equal to the cmc. Then [1] may be rearranged to give

$$[D^-]_t = \mathrm{cmc}(\delta_f - \delta_m)/(\delta - \delta_m). \qquad [2]$$

For F_3SDS in buffer at 35°, cmc = 7.52 mM, δ_f = 3.636 ppm, and δ_m = 4.789

ppm. Then $[D^-]_t$ may be found from [2] or graphically by plotting δ against $1/[D^-]_t$ for a series of known solutions and using the resulting linear graph as a calibration curve. The latter procedure was used in the experiment involving F_3STS.

From the known total number of moles of detergent in each dialysis run, S_{tm}, and the concentration in the dialysate, determined from the chemical shift, the concentration in the retentate may be calculated using

$$[D^-]_{t,r}V_r + [D^-]_{t,d}V_d = S_{tm}, \qquad [3]$$

where the additional subscripts r and d identify the retentate and dialysate, with volumes V_r and V_d. In this work V_r was equal to V_d at the beginning of each dialysis, and possible small volume changes during equilibration were neglected. If $[D^-]_f$ and $[D^-]_m$ can be determined for the retentate, then $[D^-]_b$ can be evaluated next, and with [1] δ_b can be found. To do this, we assumed that at equilibrium

$$[D^-]_{f,r} = [D^-]_{f,d} \text{ and } [D^-]_{m,r} = [D^-]_{m,d}. \qquad [4]$$

As reported by Abu-Hamdiyyah and Mysels (9) and verified in our laboratory (8), these equations are satisfied when dialysis is carried out with protein-free solutions. In the presence of protein, small corrections are required in principle, because the protein-detergent complex may produce a slight change in the cmc and because of the Donnan effect. Noting that the added buffer salts tend to reduce the magnitude of these corrections and also that validity of [4] was implicitly assumed by Reynolds and Tanford (5) in analyzing their data for protein - SDS complexes, we decided to neglect these corrections. Algebraic manipulation then provides the expression

$$\delta_b = \{[D^-]_{t,r}\delta_r - [D^-]_{t,d}\delta_d\}/\{[D^-]_{t,r} - [D^-]_{t,d}\} \qquad [5]$$

which gives δ_b in terms of the average shifts for the retentate and dialysate, δ_r and δ_d.

Measured values of S_{tm}, δ_d, and δ_r appear in Table I together with values of δ_b calculated with eq. [5]. None of the latter differs by more than 0.05

Table I. Chemical Shift and Binding Data for Sodium Trifluoroalkylsulfates and Reduced Proteins.[a]

Protein Type	Conc. (g/dl)	S_{tm} (mmoles of detergent)	Chemical Shifts (ppm) δ_d	δ_r	δ_b	$\bar{n}$ (mmoles/g)
β-Lactoglobulin A	0.480	0.1846	4.002	4.537	4.93	3.1
	0.480	0.2245	4.213	4.568	4.93	3.1
	0.480	0.2744	4.358	4.586	4.89	3.1
	0.480	0.3243	4.447	4.610	4.89	3.0
	0.480	0.3742	4.517	4.631	4.94	2.5
Bovine Serum Albumin[b]	0.496	0.2241	4.219	4.572	4.94	2.9
	0.496	0.2737	4.356	4.599	4.93	3.0
Lysozyme[c]	0.470	0.2292	4.230	4.593	4.97	3.2
β-Galactosidase[d]	0.303	0.2455	4.337	4.568	4.97	3.6
Ovalbumin[e]	0.503	0.2756	4.373	4.610	4.97	2.7
Hemoglobin[f]	0.130	0.2207	4.268	4.546	4.97	8.4
Myoglobin[g]	0.510	0.2761	4.309	4.641	4.95	3.8
	0.510	0.2761	4.327	4.645	4.98	3.5
Chymotrypsinogen A[h]	0.493	0.2761	4.342	4.621	4.95	3.4
Bovine Serum Albumin[j]	0.483	0.03533	4.565	4.785	4.93	3.2

a. All entries except those on the last line are for F_3SDS with $V_r = V_d = 5.00$ ml, ionic strength 0.050, and at 35°. Entries on the last line are for F_3STS with $V_r = 0.97$ ml, $V_d = 1.00$ ml, ionic strength 0.10 and at 40°.
b. Armour Pharmaceutical Co., Lot No. G34805.
c. 3X recrystallized and lyophilized. Schwartz-Mann, Lot No. N1178.
d. E. Coli. Worthington Biochemical Corp., Lot No. BG43E015.
e. 5X recrystallized. Nutritional Biochemical Co., Control No. 5715.
f. Bovine 2X recrystallized. Mann Research Lot No. V3553.
g. Equine. Pierce Chemical Co., Lot No. 10319-9.
h. Bovine, 0.42% intrinsic chymotrypsin. Worthington Biochemical Corp.
j. Crystallized. Armour Pharmaceutical Co., Lot No. C70710.

ppm from their average, which is 4.94 ppm. This is very near the value of δ_m given above, 4.79 ppm, and quite unlike either δ_f or the shift of 3.42 ppm reported (2) for F_3SDS bound at the initial binding sites of bovine serum albumin.

The calculation of δ_b does not require a knowledge of the protein concen-

tration or of $\bar{n}$, the number of moles of detergent bound per gram of protein. However, $\bar{n}$ can be calculated when the protein concentration is known, and the results also appear in Table I. The amount bound appears to be independent of the concentration of unbound detergent when the latter exceeds the cmc, as reported for protein-SDS complexes (5). Different proteins appear to bind roughly equal amounts of F_3SDS, with the possible exception of hemoglobin, where we suspect that the protein concentration may be in error. Excluding hemoglobin, the average $\bar{n}$ is 3.2 millimoles/g, considerably less than the value of 4.9 for protein-SDS complexes.

Evidence that the complexes formed with F_3SDS and with SDS are nevertheless at least qualitatively similar is provided by measurements of the effect of the two detergents on the specific optical rotation of lysozyme in the presence of dithioerythritol. SDS changed $[\alpha]_{233}$ from about -4.4×10^3 to about -4.97×10^3, and the effect was independent of the detergent concentration over the range of 2 to 6 mM. With F_3SDS the full effect was not obtained until the detergent concentration reached 5 mM, but the limiting value, -4.9×10^3, was quite close to that found with SDS.

Because earlier work (2) showed that F_3STS resembles ordinary SDS even more closely than F_3SDS does, we carried out a single experiment with F_3STS and reduced bovine serum albumin. The results, given in the last line of Table I, essentially duplicate those found for F_3SDS.

Although the difference in the values of $\bar{n}$ indicates that conclusions from work on protein-F_3SDS complexes must not be applied to protein-SDS complexes without due caution, our results strongly support the suggestion that the bound detergent ions form micelle-like clusters, which was put forward by Pitt-Rivers and Imbiombato to account for the solubilization of Sudan Yellow by protein-SDS complexes (4). The existence of such clusters, stabilized by interactions between the ionic headgroups and the protein, is also consistent with proton chemical shift measurements for albumin-SDS complexes (7). For F_3SDS, changes in the molecular weight or composition

of the protein do not produce significant changes in δ_b, as might have been expected if there were extensive contact between alkyl groups of the detergent and sidechains of the protein molecules.

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